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(54) **Improvements in probiotic-type products.**

(57) A probiotic-type product and method of manufacture thereof wherein non-pathogenic bacteria are grown in a liquid culture, the bacterial cells are isolated from the bulk of the fluid substrate and are killed, as by autoclaving, boiling, exposing to altered pH, or subjecting to fluctuating pressure or sound or microwaves, and the isolated killed cells are combined with a carrier to provide a solid or liquid product ready for use as an animal feed supplement.

EP 0 279 618 A2

Description

Improvements in Probiotic-type Products

This invention relates to a probiotic-type product for oral administration to or consumption by animals or possibly humans.

Known probiotic products for oral administration to animals, either specifically or by inclusion in feed, consist of fermented cultures of non-pathogenic bacteria, typically lactobacilli and/or streptococci.

Probiotics are used to improve rate of growth and feed conversion efficiency in young animals and to reduce incidence of enteritis and diarrhoea. However, the effects of such products are highly variable.

The essential mode of action of probiotics is not, in fact, understood. It is commonly thought that they enhance the numbers of non-pathogenic bacteria in the gut, which may assist digestion, possibly through enzyme production, and also increase competition with populations of potentially harmful bacteria such as Escherichia coli. However, this is not proved. In fact, the normal dosage of a probiotic is usually insufficient to add appreciably to the intestinal population and this casts doubt on the commonly believed mode of action referred to.

Most importantly, since the mode of action is not in fact sufficiently understood, meaningful quality control of probiotic products is virtually impossible.

A principal object of this invention is to provide a probiotic-type product and economical method of manufacture thereof based on improved knowledge of the source of the beneficial effects of probiotic cultures. This permits meaningful assay and hence meaningful quality control.

According to one aspect of the invention, there is provided a probiotic-type product comprising a carrier supporting killed non-pathogenic microbial cells, the killed cells having been produced by growing the non-pathogenic microbial cells in a liquid culture, isolating the said cells from the bulk of the fluid substrate and killing the cells either before or after isolation.

According to another aspect of the invention, there is provided a method of manufacturing a probiotic-type product which comprises the steps of:- a) growing non-pathogenic microbial cells in a liquid culture; b) isolating the microbial cells from the bulk of the fluid substrate; c) killing the cells, as by autoclaving or boiling or otherwise, either before or after step b); and d) combining the killed cells with a carrier to provide a product ready for use.

The microbial cells are preferably bacterial cells, although microbial cells such as yeast cells may alternatively be employed, and the cells are preferably killed after isolation from the bulk of the fluid substrate.

It will be understood that, conventionally, probiotic products are products containing live bacteria. The product in accordance with the present invention contains only killed bacteria, but being a product intended for administration or use in analogous manner to known pro biotics, i.e. administration of a bacterial culture to animals or to men, is for convenience referred to as a probiotic-type product.

The probiotic-type product of the present invention can take several forms. Thus, for example, the killed cells, in the form of a wet slurry, can have a bacteriostat added thereto for storage and administration in the wet state. Alternatively, the killed cells can be dried and included in an animal feed. Yet again, the killed cells can be absorbed in a drying agent, such as expanded mica, ready for inclusion in an animal feed.

Non-pathogenic bacterial cells which may be included in the liquid culture employed in this invention include lactobacilli and streptococci, especially the former.

The probiotic-type product in accordance with the invention has been tested on chicks in trials lasting 14 days or more. Different groups of chicks were fed a basic diet as a control diet and other diets having allegedly beneficial additives, the additive for one group being the probiotic-type product in accordance with the invention. Trials were initially carried out using a commercial broiler feed as a control diet, to assess the effects of differing allegedly beneficial additives. Additives used were live bacterial probiotics known by the Trade Marks Pronifer and Maxipro. The growth and feed conversion efficiency of groups of chicks receiving these products were compared with similar groups of chicks receiving the same originally live bacterial products, but where the products had been subject to autoclaving so as to kill the bacterial cells present in the products. It was found that substantially improved results were obtained when the live bacterial probiotics were autoclaved, thus killing all bacteria present. Further trials were then carried out in which bacterial cultures were used as additives, both as whole cultures and after centrifugal separation into the bulk liquid substrate and the cellular concentrate or residue of killed bacteria. The results were compared against the same control diet as before and also the control diet with added killed yeast cells.

The statistical results of these trials have shown that, especially after a full trial of at least 14 days, the best liveweight gain and best feed conversion ratio is to be expected from the cellular concentrate or residue containing killed bacteria.

The cellular concentrate thus has, to an unexpected extent, nutritive benefit to the growth of the animals.

Quality control is enabled firstly because the cellular content of the bacterial culture may be determined in conventional manner before autoclaving, for example colony counting techniques using plate cultures, and secondly because an index of the content is readily obtained either by dry matter estimation of the culture or the concentrated slurry or by using acid or gas production as parameters of bacterial growth.

More generally, various advantages arising from use of the probiotic-type product in accordance with the invention are as follows:- a) the total cell mass can be estimated and related to the effectiveness of the

product; b) stability is improved over conventional probiotics as no live bacteria are present; c) the low volume of the cellular concentrate enables production of a solid, powdered or granular material, when this is required for inclusion in dry animal feeds; d) liquid products can be manufactured using the killed bacterial cells, since the matter is inert (it is difficult to keep cells alive for long periods in a liquid); e) the liquid substrate is a by-product of the method of manufacture which, when used as a bulk liquid feed, is also found to possess useful nutritional properties.

A practical probiotic-type product and method of production thereof in accordance with the invention will now be described by way of example.

First, one exemplary formulation of a base culture will be given:-

Milk powder 60 g/l
Yeast extract 4 g/l
Sucrose 10 g/l
Beef and vegetable extracts 1 g/l
Vitamin B_{12} 5 mg/l
Magnesium sulphate 0.2 g/l
Manganous sulphate 0.05 g/l
'Tween 80' (Trade Mark) 1 m/l

To this may be added:-

D. Potassium H.phosphate 2.0 g/l
Sodium acetate 5.0 g/l
Tri-ammonium citrate 2.0 g/l,

more especially to act as buffers if pH is not otherwise controlled during the subsequent fermentation process.

The medium is made up to one litre with de-ionised water.

Production of the probiotic-type product is then carried out generally in accordance with the following steps:-

1. The base culture is mixed using a shearing-type mixer.

2. The culture is sterilised by autoclaving at a pressure not less than 5 p.s.i., conveniently for 15 minutes at 121 degrees C. The culture could be sterilised in other known ways, if desired, which will ensure that any contaminants are killed.

3. The culture is cooled or allowed to cool to about 41 degrees C.

4. The base culture is inoculated with a small volume of an active starter culture, more especially but not exclusively a lactobacillus culture (such as Lactobacillus fermentum) or a streptococci culture or bacillus subtilus.

5. The culture is allowed to ferment, at a temperature of about 41 degrees C, for a period of about 72 hours, whilst being subjected to gentle agitation.

6. During the fermentation step, a pH value of between 5 and 6, conveniently about 5.5, is maintained by means of NaOH and/or by means of the buffers previously referred to. If, as is preferred, pH is controlled by use of sodium hydroxide, this may be added initially, and then automatically, responsively to the output of a pH sensor.

7. During the fermentation process, gas production is monitored with a gas flowmeter, acid production is monitored by titration, and optionally cell production may be monitored by cell counting.

8. At the end of the fermentation period, the fermented culture is cooled or allowed to cool to ambient temperature. Settling occurs during this period of cooling.

9. The supernatent liquid is then syphoned off. This leaves a residue in the form of a slurry containing the cells produced by fermentation.

10. The slurry residue is mixed by a shearing-type mixer.

11. The slurry is autoclaved at a pressure of at least 5 p.s.i., typically about 121 degrees C for 20 minutes. Autoclaving can be carried out at a higher pressure (and temperature) if desired. The result is a slurry with a high content of killed bacterial cells.

12. The killed cell slurry is again mixed by a shearing-type mixer.

13. The killed cell slurry is then dried, as by freeze drying or addition of a bacteriostat and mixing with a drying agent. Again, spray drying or other drying by use of heat may be employed instead.

14. The resulting killed cell residue in dried condition is combined with a carrier and constitutes a probiotic-type product ready for use, more especially as an animal food supplement.

It is alternatively possible to produce a liquid product, again suitable for use as an animal feed supplement, by mixing the wet residue of step 12 or the dried residue of step 13 with a liquid carrier together with a bacteriostat, although a solid product is preferred for inclusion in dry feed.

A cellular concentrate containing only autoclaved cells of Lactobacillus fermentum and added to a basic control diet has been tested on broiler chicks over a period of 0 to 21 days of age with the following results. In the table, the column headings designate the number of parts by weight of cellular concentrate added to one

million parts of weight of control feed, and the successive rows in the table indicate weight gain (WG) in grams, weight gain as a percentage of control (WG%), the feed conversion ratio (FCR) and the feed conversion ratio as a percentage (FCR%), taking the FCR for control as 100.

Table

	0	50	100	200	400
WG	335.25	381.08	401.81	390.52	371.36
WG%	100	113.67	119.85	116.49	110.77
FCR	2.963	2.216	2.096	2.139	2.541
FCR%	100	74.79	70.74	72.19	85.75

A prototype commercial food additive produced substantially by the method hitherto described has been tested on broiler chicks over a period of 0 to 18 days. The control diet used for this test was a commercially available mix containing wheat (63.4), soya extract (25.0), full fat soya (3.6), dicalcium sulphate (2.0), DL-Methionine (0.22), salt (0.2), Minvite 204 (0.5) and soya bean oil (4.8), with added choline chloride (0.5), the figures being percentages.

In the following table, the column headings indicate grams/ton of additive employed, first when the fermented slurry residue was autoclaved and second when this residue was boiled.

Table

	Control	Autoclaved Approx.30 g/tonne	Boiled Approx.30 g/tonne
WG	360.17	381.75	376
WG%	100	105.99	104.04

This result indicates that boiling is nearly as effective as autoclaving as a means of processing the bacterial cells.

It is also important to note that trials with broiler chicks have been conducted in which WG and FCR were compared when a) the cellular concentrate was used as a food additive, b) the bulk liquid substrate emerging as a by-product of the process was used as an additive, c) the bulk fermented fluid autoclaved but without separation of the bulk fluid substrate was used as an additive, and d) the bulk dried culture without separation and autoclaving was used as an additive. These trials have shown that a substantially improved WG and FCR are obtained with the cellular concentrate as compared with any of the other additives. It is therefore to be understood that process step 9 of the above-described method of production, in which the bulk liquid substrate is syphoned off or otherwise removed, is an essential step in production of the animal feed additive in accordance with the invention. Clearly, step 11 of the process is also essential.

The identity of the substance obtained from killed microbial cells and having considerable beneficial effect on animal growth and feed conversion efficiency is not yet known. However, tests for antibiotic activity have proved negative.

It will be understood that various modifications of the above-described method of production are possible within the scope of this invention. For example, the microbial cells may be killed, instead of by autoclaving or boiling, by such methods as irradiation, sonication, pressure fluctuation and pH alteration.

It should also be made clear that the product of the invention is useful for administration to animals other than chicks, such as pigs and other monogastrics, as well as ruminants.

Claims

1. A probiotic-type product comprising a carrier supporting killed non-pathogenic microbial cells, the killed cells having been produced by growing the non-pathogenic microbial cells in a liquid culture, isolating the said cells from the bulk of the fluid substrate and killing the cells either before or after isolation.

2. A product according to claim 1, wherein the non-pathogenic cells are bacterial cells including lactobacilli and/or streptococci.

3. A product according to claim 1 or claim 2, comprising a wet slurry containing the killed cells and with a bacteriostat added thereto.

4. A product according to claim 1 or claim 2, comprising an animal feed additive incorporating the killed cells in a dried state.

5. A method of manufacturing a probiotic-type product for use as an animal feed supplement, which comprises the steps of:- e) growing non-pathogenic microbial cells in a liquid culture; b) isolating the cells from the bulk of the fluid substrate; c) killing the cells either before or after step b); and d) combining the killed cells with a carrier to provide a product ready for use.

6. A method according to claim 5, wherein the cells are killed by autoclaving, by boiling, by exposing to acid or alkali, or by exposure to fluctuating pressure, as by French pressing, or by sonication.

7. A method according to claim 5 or claim 6, wherein the cells are killed after separation of the bulk fluid substrate.

8. A method according to any of claims 5 to 7, wherein the killed cells are dried prior to combination with the carrier.

9. A method according to any of claims 5 to 8, wherein the killed cells are combined with a bacteriostat to form a liquid product.

10. A method according to any of claims 5 to 9, including the step of estimating the cellular content of the killed cell concentrate to enable quality control.